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# The role of ingested enzymes in the digestive processes of insects

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## Introduction

It has been recognized since the classic investigations of Buchner (1921, 1928) and Cleveland (1924) that microbial symbionts can play an important role in the digestive processes of insects. Permanent populations of hindgut protozoa are responsible for cellulose digestion in the lower termites (Noirot & Noirot-Timothée, 1969; Honigberg, 1970; Lee & Wood, 1971; Breznak, 1975, 1982; LaFage & Nutting, 1978; O'Brien & Slaytor, 1982) and in the wood roach, *Cryptocercus punctulatus*, (Cleveland, Hall, Sanders & Collier, 1934), while permanent populations of hindgut bacteria contribute to cellulose digestion in the American cockroach, *Periplaneta americana* (Bignell, 1977; Cruden & Markovetz, 1979) as well as in the rhinocerus beetle, *Oryctes nasicornis* (Bayon & Mathelin, 1980). My co-workers and I have recently discovered a different mechanism by which insects accomplish the digestion of refractile dietary components, namely by the acquisition through ingestion of fungal enzymes active against plant cell wall polysaccharides (Martin & Martin, 1978, 1979; Martin, 1979). This review summarizes the results of our investigations of the significance of acquired fungal enzymes in the digestive processes of the fungus-growing termites, the siricid woodwasps, and the fungus-growing ants, and will conclude with a brief survey of other studies which suggest that the acquisition of a digestive capacity through the ingestion of a set of enzymes may be a fairly widespread phenomenon of considerable biological importance. Since acquired fungal enzymes have been shown to contribute to cellulose digestion in several species of wood-feeders, and seem likely to be implicated in other insects with diets high in

cellulosic materials, it is appropriate to give a brief description of the enzymatic basis for cellulolysis in fungi.

*The cellulolytic enzymes of fungi*

In fungi, cellulose digestion is accomplished by a 'cellulase complex' that includes three major classes of hydrolytic enzymes (Table 6.1): endoglucanases ($C_x$-cellulases), cellobiohydrolases ($C_1$-cellulases), and cellobiases (Reese & Mandels, 1971; Bailey, Enari & Linko, 1975; Wood & McCrae, 1979; Ghose, Montenecourt & Eveleigh, 1981). The digestion of native cellulose is believed to be initiated when endoglucanases attack isolated amorphous regions of the predominantly crystalline cellulose, creating nicks in the linear cellulose chains. The cellobiohydrolases attack at these 'nick sites', liberating cellobiose, exposing additional potential sites for attack by the endoglucanases, and generally disrupting the highly ordered structure of the cellulose matrix. The continued combined action of the $C_x$- and $C_1$-cellulases results eventually in the complete degradation of the original cellulose and the production of cellobiose and a mixture of soluble linear oligosaccharides of varying chain lengths. The cellobiose, which is a potential inhibitor of both the $C_1$- and $C_x$-cellulases, is hydrolyzed to glucose by cellobiase, and the various oligosaccharides are further degraded, ultimately to glucose, by the action of both the $C_x$-cellulases and the cellobiases. Thus, the utilization of cellulose is dependent upon the concerted and synergistic action of three types of enzymes. If any one of the three is missing, cellulose digestion cannot proceed.

**The fungus-growing termites**

The fungus-growing termites (Termitidae, subfamily Macrotermitinae) forage for food in seasoned structural timber, fence posts, sound dead wood, stumps, grass, and herbivore dung (Coaton & Sheasby, 1972). They are higher termites which lack the assemblage of xylophagous protozoa that provide the lower termites with the cellulolytic enzymes they require in order to digest cellulose. These termites have long fascinated biologists because of their symbiotic association with fungi that grow in their nests on structures referred to as 'fungus combs' (Sands, 1969). The surface of the comb is covered with a sparse growth of mycelium and numerous white spheres or nodules, 0.5 to 2 mm in diameter (Fig. 6.1). These nodules are the conidia or conidiophores of a fungus, *Termitomyces* sp, believed to be restricted to the nests of the Macrotermitinae. The mycelium is a mixture of

Table 6.1. *Enzymes involved in cellulose depolymerization by fungi*

| *Enzyme (Alternate designations)* | *Mode of action and products* | *Substrates* |
|---|---|---|
| The cellulase complex | A combination of the three categories of enzymes designated below, which brings about the complete digestion of native cellulose to glucose. | Microcrystalline cellulose powder, Avicell®, cotton, and filter paper. |
| 1,4-β-D-Glucan 4-glucanohydrolase (EC 3.2.1.4) (Endo-β-1,4-glucanase) (Endoglucanase) (Carboxymethylcellulase) (CMCase) ($C_x$-Cellulase) | Random attack on β-1,4-glucosidic bonds, generating transient cellodextrins, cellobiose, and glucose | CMC and other soluble derivatives of cellulose, phosphoric acid swollen cellulose, and cellodextrins (increasing activity with increasing chain length). No activity toward crystalline cellulose. Hardly any activity toward cellobiose |
| 1,4-β-D-Glucan cellobiohydrolase (EC 3.2.1.91) (Cellobiohydrolase) ($C_1$-cellulase) | Removal of cellobiose units from the non-reducing end of a β-1,4-glucan chain by attack on penultimate β-1,4-glucosidic bonds | Microcrystalline cellulose powder, Avicel®, cotton, swollen cellulose, and dextrins (increasing activity with increasing chain length). Limited activity toward CMC. |
| 1,4-β-D-Glucoside 4-glucohydrolase (EC 3.2.1.21) (β-D-Glucosidase) (Cellobiase) | Hydrolysis of the β-1,4-glucosidic bond of cellobiose to generate glucose | Cellobiose, other β-linked disaccharides of glucose, and cellodextrins. No activity toward cellulose. |

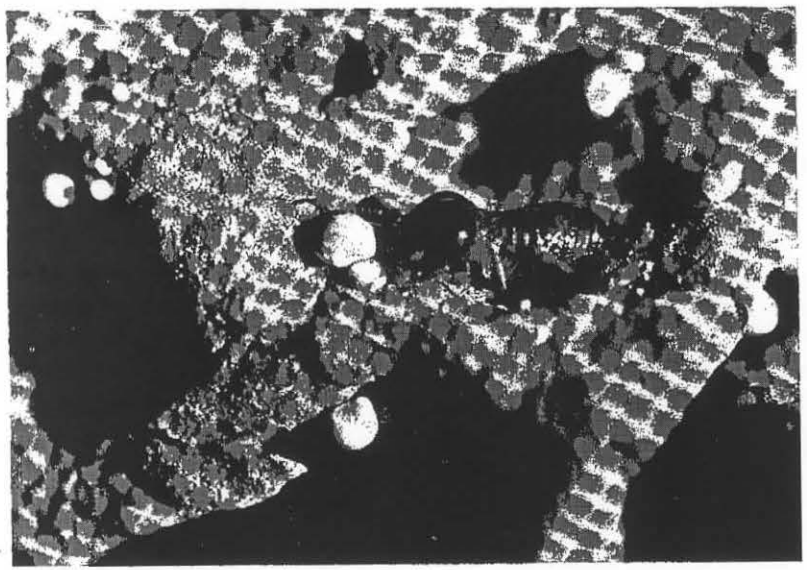

Fig. 6.1. *Macrotermes natalensis* worker on fungus comb bearing conidiophores of *Termitomyces* sp. (Photograph by Dr Robin Crewe, Dept. of Zoology, University of the Witwatersrand, Johannesburg, South Africa.)

*Termitomyces* and various xylariaceous fungi. The comb is constructed of small fragments of plant tissue in which intact cellular structure is still evident in scanning electron micrographs (Rohrmann, 1978; Wood, 1978). The termites rapidly starve to death on a diet composed exclusively of sound wood or filter paper, but survive for extended periods of time if they are also provided with a fragment of fungus comb complete with nodules (Sands, 1956; Ausat *et al.*, 1960). Survival is not prolonged if the fragment of fungus comb provided for the termites has been sterilized (Sands, 1956). These studies demonstrate that there is something in the live, intact fungus combs which is crucial to the nutritional well-being of the termites. The latter are known to consume both the *Termitomyces* nodules and the comb material (Grassé & Noirot, 1957; Alibert, 1964; Batra, 1975).

Martin & Martin (1978, 1979) showed that the midgut of the fungus-growing termite, *Macrotermes natalensis*, contains $C_1$-cellulases, $C_x$-cellulases, and $\beta$-glucosidases (presumably including cellobiases), whereas the paunch, which is the name given to the enlarged anterior portion of the hindgut where numerous bacteria reside, contains



Table 6.2. *Cellulase complex and $C_x$-cellulase activity of* Macrotermes natalensis *workers and their fungus gardens*

| | *Units of enzymatic activity (per termite gut section or per mg of fungus or comb material)* | |
|---|---|---|
| | *Cellulase complex* | *$C_x$-cellulase* |
| Midgut (tissue + contents) | 0.45 ± 0.05 (7) | 1.19 ± 0.16 (6) |
| Paunch (tissue + contents) | 0.04 ± 0.01 (5) | 0.62 ± 0.08 (5) |
| Rectum (tissue + contents) | 0.05 ± 0.02 (3) | 0.50 ± 0.17 (3) |
| Midgut (tissue) | 0.00 ± 0.00 (3) | 0.33 ± 0.14 (3) |
| Salivary glands | 0.01 ± 0.01 (5) | 0.72 ± 0.14 (7) |
| Fungus nodules | 0.53 ± 0.05 (4) | 1.60 ± 0.46 (6) |
| Comb material | 0.01 ± 0.01 (3) | 0.04 ± 0.03 (3) |

*Note:* Each value is the mean ± SEM for the number of replicates indicated in parenthesis. A unit of activity is the amount of enzyme required to liberate 1 micromole of maltose equivalents per hour under the conditions of the assay (37 °C, pH 5.0, incubation volume 1.0 ml). Substrates were microcrystalline cellulose and CMC.

*Source:* From Martin & Martin, 1978, 1979.

$C_x$-cellulases and $\beta$-glucosidases but only a barely detectable trace of activity attributable to the presence of $C_1$-cellulases (Table 6.2). These results clearly identify the midgut as a major site of cellulose digestion in this insect, especially the early stages of cellulose digestion when the $C_1$-enzymes are so critical. Extracts of the salivary glands and the midgut tissue actively degrade carboxymethylcellulose (CMC) but not microcrystalline cellulose, demonstrating that the insects are able to synthesize $C_x$-cellulases but not $C_1$-cellulases. However, an extract of the fungus nodules actively digests microcrystalline cellulose and contains high levels of $C_x$-cellulase activity (Table 6.3), demonstrating that $C_1$-cellulases are present in the *Termitomyces* nodules. In contrast to the fungus nodules, the comb itself is virtually devoid of enzymatic activity. These findings suggested to us the possibility that the *Termitomyces* nodules might be the source of the $C_1$-cellulases detected in the midgut fluids of the termite workers. Further evidence in support of this hypothesis was obtained when we demonstrated that after 72 hours the level of activity toward microcrystalline cellulose decreased by 40 to 50% in the midguts of termites provided only with water or with fungus comb from which the nodules had been removed, while the level of activity remained at 100% of the value measured in termites collected

Table 6.3. *Enzymatic activity toward various polysaccharides of extracts of midguts (tissue + contents) of* Sirex cyaneus *larvae and of extracts of cultures of their symbiotic fungus,* Amylostereum chailletii

| | *Units of enzymatic activity per milligram dry weight of dissolved solids in extract* | | | | | |
|---|---|---|---|---|---|---|
| | *Amylase* | Cellulase *complex* | *$C_x$-cellulase* | *Laminarinase* | *Pectinase* | *Xylanase* |
| Larvae collected from natural galleries in a balsam fir log | 6.06 ± 2.62 (5) | 0.33 ± 0.13 (5) | 8.86 ± 6.20 (5) | 14.23 ± 8.91 (5) | 8.47 ± 5.30 (5) | 11.59 ± 7.21 (5) |
| Larvae cultured for one week on balsam fir chips permeated by *A. chailletii* mycelium | 4.18 ± 1.30 (5) | 0.48 ± 0.01 (5) | 20.17 ± 0.59 (5) | 12.91 ± 3.14 (5) | 11.03 ± 1.11 (5) | 33.41 ± 3.97 (5) |
| Larvae cultured for one week on sterile balsam fir chips | 2.36 (1) | 0.0 (1) | 0.09 (1) | 1.62 (1) | 0.97 (1) | 1.39 (1) |
| Balsam fir chips permeated by *A. chaelletii* mycelium | 2.54 ± 0.00 (5) | 0.37 ± 0.00 (5) | 1.58 ± 0.65 (5) | 5.65 ± 2.12 (5) | 1.50 ± 0.74 (5) | 6.78 ± 2.53 (5) |
| Sterile balsam fir chips | 0.02 ± 0.01 (5) | 0.0 (5) | 0.0 (5) | 0.02 ± 0.01 (5) | 0.0 (5) | 0.0 (5) |
| *A. chailletti* growing on microcrystalline cellulose suspended in a chemically defined liquid medium | 9.48 ± 0.26 (5) | 0.33 ± 0.03 (5) | 7.45 ± 0.33 (5) | 8.47 ± 0.38 (5) | 9.76 ± 0.46 (5) | 9.24 ± 0.33 (5) |

*Note:* Each value is the mean ± SEM for the number of replicates indicated in parenthesis. A unit of activity is the amount of enzyme required to liberate 1 micromole of maltose equivalents per hour under the conditions of the assay (37°, pH 5.0, incubation volume 1.0 ml). Substrates were potato $\alpha$-amylose, microcrystalline cellulose, CMC, seaweed laminarin, citrus pectin, and larchwood xylan.

*Source:* From Kukor & Martin, 1983.



from a nest if the termites were provided with a supply of nodules. $C_x$-activity remained at the normal level in termites which were not allowed to consume the *Termitomyces* nodules, suggesting that the decrease in activity toward microcrystalline cellulose in the termites deprived of access to the nodules was due to a decrease in the amount of $C_1$-cellulase present.

Finally, by the use of the technique of isoelectric focusing, we confirmed the identity of the $C_1$-cellulases in the nodules and the $C_1$-cellulases in the termites' midgut. Both the nodule and the midgut extracts contain two separate $C_1$-cellulases. The major enzyme from both sources has an isoelectric pH between 3.90 and 4.05, while the minor enzyme from both sources has an isoelectric pH in the range 4.20 to 4.35. Our isoelectric focusing studies also revealed that some of the $C_x$-cellulase activity detected in the midgut of the termite is due to enzymes originating from the ingested fungus nodules. These studies demonstrated that the ability to digest cellulose by *M. natalensis* is dependent upon the acquisition of critical enzymes, the $C_1$-cellulases, through the consumption by the termites of their fungal symbiont.

Abo-Khatwa (1978), studying the closely related termite *M. subhyalinus*, obtained comparable results and arrived at an identical interpretation.

**The siricid woodwasps**

More recently we have investigated the digestive processes in the larvae of the siricid woodwasp, *Sirex cyaneus* (Siricidae), another wood-feeder that is associated with a fungal symbiont (Francke-Grosmann, 1967; Kukor & Martin, 1983). The larvae develop in timber in galleries lined with the mycelium of a fungus introduced into the wood along with the egg at the time of oviposition. *Sirex* larvae can be grown normally on a pure culture of the symbiotic fungus, *Amylostereum chailletii*, but fail to survive in galleries devoid of fungal growth (Cartwright, 1929; Stillwell, 1966). The normal larval diet is a mixture of wood and the mycelium of their symbiotic fungus (Buchner, 1928, 1965).

An extract of whole guts dissected from larvae collected from natural galleries in a balsam fir log catalyzes the degradation of both microcrystalline cellulose and CMC (Table 6.3). Although activity toward microcrystalline cellulose is low compared to activity toward CMC and several other polysaccharides, the observed level is by no means a marginal one barely exceeding the lower limits of detection. The assay results provide

an unambiguous demonstration that the gut fluids of this insect contain both $C_1$- and $C_x$-cellulases. Müller (1934) established the capacity of siricids to digest cellulose by determining that 22 and 31% of the cellulose in fir and spruce wood is assimilated during passage through the guts of *S. gigas* and *S. phantoma* respectively. These two species also assimilate significant quantities of ingested hemicellulose.

Experiments in which the diet of *S. cyaneus* is manipulated provide strong evidence that the $C_1$- and $C_x$-cellulases, as well as enzymes active against xylan, laminarin and pectin, are acquired from ingested fungal tissue (Table 6.3). The larvae can be maintained quite satisfactorily on a diet of balsam fir wood chips on which the symbiotic fungus has been allowed to grow for about a month. The larvae feed actively and appear to be normal. Their digestive fluids contain the same digestive enzymes as larvae collected from natural galleries, and at roughly comparable levels of activity. However, if the larvae are put on a diet of sterile balsam fir chips, they fare very badly. Mortality is high, and after a week enzymatic activity toward cellulose, CMC, xylan, laminarin, and pectin has declined to very low levels. Amylose is the only substrate toward which enzymatic activity remains high in larvae on a diet of sterile wood chips. Enzymes active against all of these substrates are present in the fungal symbiont of *S. cyaneus*, and are readily detectable in balsam wood chips on which the fungus is growing (Table 6.3).

The fungal origin of the enzymes responsible for $C_x$-cellulase and xylanase activity in the gut fluids of the woodwasp larvae was confirmed by establishing the identities of the gut and fungal enzymes using the techniques of isoelectric focusing and chromatofocusing. The $C_x$-cellulases and the xylanases present in a gut extract and in a liquid culture medium in which *A. chailletii* had been growing were purified by a protocol involving concentration, dialysis, ion exchange chromatography, gel permeation chromatography and chromatofocusing. $C_x$-cellulase and xylanase activity eluted in comparable fractions during the purification of the insect and fungal extracts. Both the larval midgut and the fungus culture extracts yielded two major $C_x$-cellulases and three major xylanases. The first cellulase from both sources eluted from the preparative chromatofocusing column at pH 4.9, and the second cellulase at a pH below 4.0. The first xylanase eluted at pH 5.7, the second at pH 5.3, and the third at pH 4.5. A further comparison of insect and fungal enzymes isolated from the chromatofocusing columns was made by subjecting the cellulase and xylanase fractions to analytical isofocusing on ultrathin polyacrylamide gels. This procedure revealed



that several proteins were present in each of the cellulase and xylanase fractions. We did not determine whether the observed protein multiplicity reflected enzyme multiplicity or simply incomplete purification. However, the point of overriding importance is that the patterns seen in each of the cellulase and xylanase fractions from the fungal extract are identical with those seen in the comparable fractions derived from the insect extract. These experiments demonstrate that the $C_x$-cellulases and xylanases present in the gut of *S. cyaneus* larvae are identical to the enzymes produced by the fungus, *A. chailletii*, and demonstrate that the woodwasp larvae, like the fungus-growing termites, acquire essential digestive enzymes by the ingestion of their fungal symbiont.

The feeding experiments summarized in Table 6.3 also strongly suggest that enzymes active against pectin and $\beta$-1,3-glucans similar to laminarin are acquired fungal enzymes. That possibility has not yet been confirmed by direct comparisons of the gut and fungal enzymes, however.

In 1928, Prof. Paul Buchner, in discussing the siricid–fungus symbiosis, wrote the following: 'Man hat den Eindruck, dass die auf solche Weise aufgenommene Menge Pilzubstanz der Larve nicht genügen kann und ich mochte die Vermutung aussprechen, dass hier eine ganz neue Variante der Ambrosiazucht vorlieght, bei der es dem Insekt vielleicht weniger auf die Substanz des Pilzes ankommt, als auf die Bestandteile des Holzes lösenden Nahrungsenzyme, die es sich mit den Mycelstücken zuführt, und die ihre dem Tier abgehende Wirkung auf das Holz auch im Darmlumen geltend machen.'[1] This passage apparently went unnoticed by workers interested in symbiosis and insect–microbial interactions. We are pleased, honoured, and slightly humbled to find that our research conducted half a century later has verified the prescient speculation of this pioneer in the field of insect–microbial interactions.

### The fungus-growing ants

The fungus-growing ants (Formicidae, subfamily Myrmicinae, tribe Attini) provide yet another variant to the role of acquired enzymes in the biology of an insect that is obligatorily associated with a fungal symbiont (Martin, Gieselmann & Martin, 1973; Boyd & Martin 1975*a*,

[1] 'One has the impression that the various fungal constituents are (dietically) insufficient for the larvae. I suggest that this represents an entirely new aspect of the Ambrosia cultivation in which the insect utilizes little of the fungus other than the range of wood-degrading enzymes. These are ingested with mycelial fragments and are active on wood in the gut lumen together with the insects' enzymes.'

*b*; Martin, Boyd, Gieselmann & Silver, 1975). In addition to being highly active against protein, the gut fluids of these ants are also active against a number of polysaccharides, including starch, CMC, pectin, xylan and chitin. We suspect that most, if not all, of these enzymes are acquired by the ants when they ingest the fluids present in the mycelium of their fungal symbiont (Fig. 6.2). We have established unequivocally that the proteolytic enzymes in the gut fluids of *Atta texana* are of fungal origin.

The proteolytic activity of the gut fluid is due to the presence of three enzymes, one serine proteinase and two metalloendoproteinases. A serine proteinase and two metalloendoproteinases are also present in an extract of the mycelial mat which results when the ants' fungal symbiont is cultured in a defined liquid medium. The fungal enzymes are not secreted into the culture medium to any significant extent. A comparison of molecular weights, substrate specificities, inhibition characteristics, pH-activity relationships and electrophoretic mobilities established the identity of the fungal enzymes and the enzymes present in the ants' gut fluids.

Unlike the fungus-growing termites and the siricid woodwasps, the food of the fungus-growing ants does not include highly refractile polysaccharides. Fungal tissue, or the fluids derived therefrom, is the exclusive food of the larvae and a minor component of the adults' diet, which consists largely of plant juices (Quinlan & Cherrett, 1979). Consequently, it does not seem likely that the ants would be dependent upon acquired fungal enzymes for the processing of ingested nutrients, and we are aware of no information which suggests a critical role for the fungal enzymes in the digestive processes occurring in the ants' digestive tracts. However, we have shown that the acquired fungal enzymes survive passage through the midgut, that they are concentrated in the rectum, and are still present and active in the faecal material, which plays a vital role in the fungus-culturing activities of these ants. The faecal fluid is regularly applied to the freshly cut leaf fragments which are used as the substrate on which *Atta* grows its fungal symbiont (Wheeler, 1907; Weber, 1972). We have shown that the fungal symbiont of *A. texana* grows very slowly in a culture medium in which the nitrogen is present in the form of polypeptides, that it grows rapidly if nitrogen is supplied as a mixture of amino acids, that it produces but does not secrete digestive proteinases, and that the growth rate of the fungus in a culture medium containing polypeptides as the sole nitrogen source is greatly increased by the addition of the ants' faecal material or



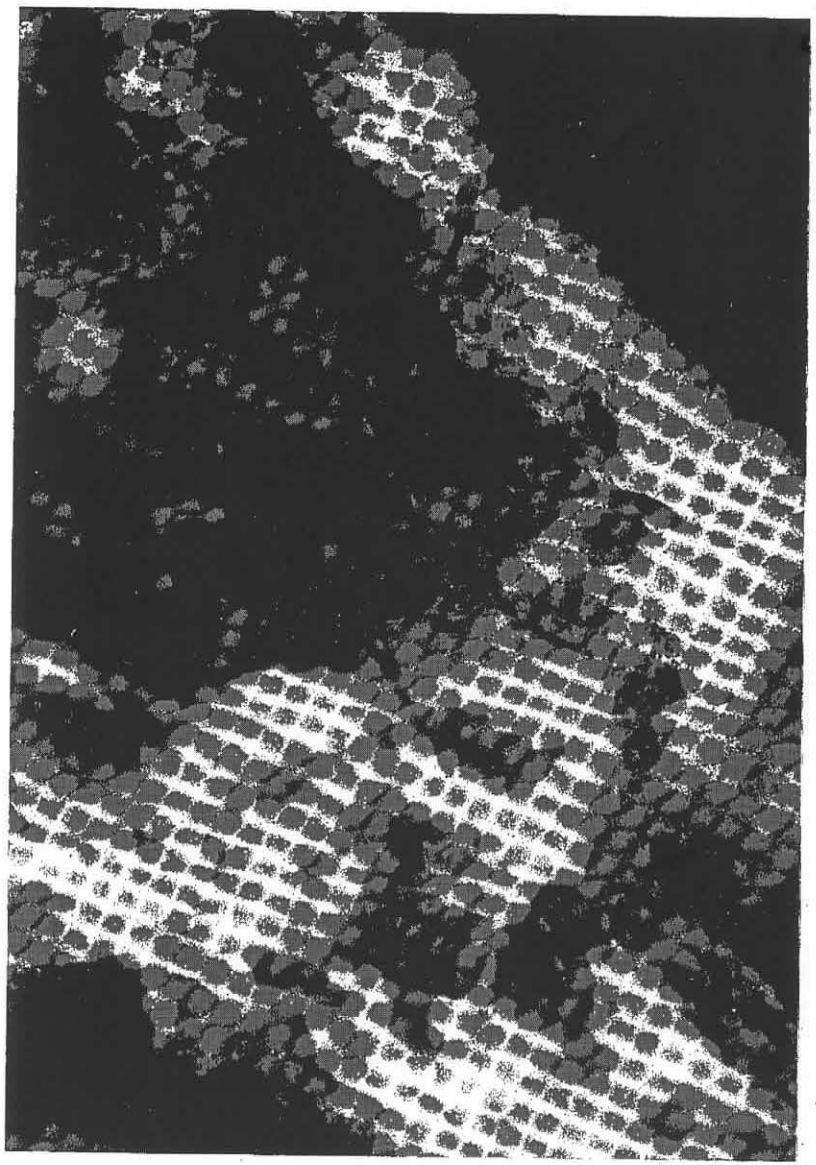

Fig. 6.2. *Atta colombica* worker on fungus garden. (Photograph by Mr David Bay, Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan, USA.)

the proteolytic enzymes it contains (Boyd & Martin, 1975*b*). The application of the ants' faecal material to the freshly collected leaf fragments can, therefore, be viewed as a mechanism for increasing the initial growth rate of fungus by augmenting the enzymatic capacity of the new inoculum to degrade protein in the leaf tissue used as substrate. In addition, the pectinases present in the faecal material should foster rapid initial growth by degrading the intercellular middle lamella, thereby facilitating the penetration and ramification of the fungal hyphae in the leaf tissue.

Copious enzyme production and rapid hyphal penetration are two major factors which contribute to competitive ability in a fungus. Defined within the context of the adaptive strategies of the fungus, the feeding and defaecation behaviour of the adult ants can be viewed simply as a mechanism that has replaced the process of enzyme secretion. However, the ants achieve a distribution of enzymes more conducive to rapid initial growth than would result from the direct secretion of the enzymes by the fungus, since the ants feed in mature portions of the garden and transport enzymes produced there in abundance to the site of inoculation, where only small quantities of enzymes can be produced by the small mycelial fragment placed there by the ants. In summary then, in this truly remarkable symbiosis the ants acquire digestive enzymes from their fungal partner, only to return them in a manner which enhances fungal fitness.

**Other examples of enzyme acquisition through ingestion**

At the present time it is not clear whether the acquisition of enzymes through ingestion is a rare phenomenon which we have fortuitously encountered because of our interest in several spectacular, but highly atypical, coevolved symbiotic associations between insects and fungi, or whether it is a common phenomenon with broad biological significance. There is certainly no information which precludes the involvement of acquired enzymes in the digestive processes of other species of higher termites or of the wood-feeding buprestid (Schlottke, 1945), anobiid (Müller, 1934; Norman, 1936; Parkin, 1940) and cerambycid (Ripper, 1930; Mansour & Mansour-Bek, 1934; Müller, 1934; Parkin, 1940; Mishra & Singh, 1978) beetles, cellulose-digesting insects which are often presumed to produce their own cellulases, even though evidence in support of this presumption is usually permissive at best. There are scattered reports which suggest that the ingestion of enzymes may be important in organisms other than wood-feeders. This review



will close with a summary of all of the other studies known to us which suggest that the ingestion of enzymes produced by another organism might be a widely occurring mechanism for acquiring a digestive capability.

Low levels of cellulase activity have been reported in many species of carnivorous marine and freshwater invertebrates (Araki & Giese, 1970; Kristensen, 1972; Monk, 1976), and Araki & Giese (1970) suggested that this activity may be due to prey enzymes. A similar origin has been proposed for low levels of cellulase activity in the gut fluids of several fish (Niederholzer & Hofer, 1979). It has not been suggested that these enzymes serve any adaptive function in the digestive processes of the predator species, but the observations at least underline the possibility that ingested enzymes can survive and remain active for extended periods of time following ingestion.

An adaptive role for ingested enzymes in several species of insects other than wood-feeders has been postulated. Applebaum (1964) reported the presence of a $\beta$-amylase of dietary origin along with an $\alpha$-amylase of insect origin in the gut fluids of *Tenebrio* larvae reared on wheat. Since the insect's own $\alpha$-amylase is inhibited by a substance in the wheat, starch digestion is presumably brought about entirely through the action of the acquired $\beta$-amylase. More recently Grogan & Hunt (1979) have suggested that protein digestion in adult honey bees is accomplished at least in part by proteolytic enzymes derived from pollen. Powning & Davidson (1979) have noted the bacteriolytic action of the gut fluids of larvae of the bean weevil *Acanthoscellides obsoletus*, and have proposed that this lytic activity is derived in part from the seeds they feed upon. The possibility that acquired enzymes might contribute to an organism's defenses against bacterial infections and aid in the detoxification of allelochemics is an important one deserving further consideration. In this context, the capacity of the terrestrial isopod *Oniscus asellus* to metabolize aromatic compounds has been attributed to ingested bacteria or bacterial enzymes (Kaplan & Hartenstein, 1978).

Detritus-feeding invertebrates would seem to be a group likely to include species that could benefit from the ingestion of microbial enzymes. Detritus consists of dead plant tissue of low nutritive value along with varying amounts of associated bacterial cells and fungal tissue. Numerous studies have demonstrated a preference on the part of detritivores for litter which supports a rich culture of microorganisms, especially fungi (Berrie, 1976; Anderson & Sedell, 1979; Cummins &

Klug, 1979). The preference for microbially colonized substrate has usually been interpreted as an ability of detritivores to select high quality over low quality food. Microbial tissue is more nutritious and more digestible than dead plant tissue, which is typically low in nitrogen and high in cell wall constituents. However, it is also possible that detritivores might benefit from selecting substrate supporting fungal growth because it provides fungal enzymes which are more effective than the invertebrate's own enzymes in degrading refractile components of the plant tissue. Low levels of $C_x$-cellulase activity have been detected in detritus-feeding stonefly nymphs (Plecoptera) (Martin, Martin, Kukor & Merritt, 1981; Sinsabaugh, Benfield & Linkins, 1981), caddisfly larvae (Trichoptera) (Bjarnov, 1972; Monk, 1976; Martin, Kukor, Martin, Lawson & Merritt, 1981), and crane fly (Sinsabaugh *et al.*, 1981), chironomid (Bjarnov, 1972) and sciarid (Terra, Ferreira & deBianchi, 1979) larvae (Diptera). On the basis of a correlation between the level of $C_x$-activity in the gut and the presence or level in the food, it has been proposed that acquired microbial enzymes are responsible at least in part for activity in the terrestrial isopod *Philoscia muscorum* (Hassal & Jennings, 1975), the aquatic amphipod *Gammarus fossorum* (Bärlocher, 1982), and the aquatic nymphs of the stonefly *Pteronarcys proteus* (Benfield, personal communication). The role of ingested microbial enzymes in the nutrition of detritus-feeding invertebrates is a subject deserving further investigation at this time.

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